PB90-103292

Health Effects Division Standard Evaluation Procedure Metabolism in Food Animals: Qualitative Nature of the Residue

(U.S.) Environmental Protection Agency, Washington, DC

8 Aug 89

U.S. Department of Commerce
National Technical Information Service
NTIS

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO.	2.	3. Recipient's Accession No. PB90 103292/AS
4. Title and Subtitle: Health Effects Division: Standard Evaluation Procedures Metabolism in Food Animals: Qualitative Nature of Residue			5. Report Date: 8/8/89 6.
7. Author(s): M.J. Nelson, Ph.D, F.D. Griffith, Jr., M.S.			8. Performing Organization Rept. No.: 540/09-89-061
9. Performing Organization Name and Address: U.S. Environmental Protection Agency OPP/HED/H7509C 401 M Street, S.W. Washington, D.C. 20460			10. Project/Task/Work Unit No. 11. Contract(C) or Grant(G) No. (C) (G)
12. Sponsoring Organization Name and Address: Same as #9			13. Type of Report & Period Covered 14.

15. Supplementary Notes

16. Abstract (Limit: 200 words)

Metabolism in Food Animals: Qualitative Nature of Residue studies are to define qualitatively these residues when livestock have been exposed to pesticides either by ingestion of treated feeds, direct exposure, or premise use. If a pesticide use could result in residues in edible tissues of livestock (including poultry), in milk, or in eggs, petitioners for tolerances are required by 40 CFR 158.240 to submit animal metabolism studies. These data are used to determine the total toxic residue (the residue considered to be in need of regulation) in tissues, milk, and eggs.

This Standard Evaluation Procedure (SEP) is designed to aid Dietary Exposure Branch scientists in their evaluation of animal metabolism studies. Residue Chemistry, and further guidance for petitioners is in the residue chemistry Data Reporting Guidelines.

17. Document Analysis a. Descriptors

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

18. Availability Statement:	19. Security Class (This Report)	21. No. of Pages: 17
	20. Security Class (This Page)	22. Price

(See ANSI-Z39.18) See Instructions on Reverse OPTIONAL FORM 272 (4-77)
(Formerly NTIS-35)

540/09-89-061
PB90-103292

HEALTH EFFECTS DIVISION

STANDARD EVALUATION PROCEDURE

Metabolism in Food Animals: Qualitative Nature of the Residue

Prepared by:

Karl H. Arne, Ph.D.

Branch Coordinator:

M. J. Nelson, Ph.D.

United States Environmental Protection Agency
Office of Pesticide Programs
Washington, D.C. 20460

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL INFORMATION SERVICE
SPRINGFIELD, VA. 22161

TABLE OF CONTENTS

	Page
TABLE OF CONTENTS	1
INTRODUCTION	2
DATA EVALUATION	
When Are Metabolism Studies Needed for Food Animals?	2
Data Requirements	
Information Concerning the Test Material	3
Information Concerning Animals	4
Dosing	4
Sample Collection	5
Quantitation of Activity	5
Extraction of Activity	6
Characterization and Identification of Activity	7
Raw Data	9
Expression of a Tolerance	9
WRITING A REVIEW	10
Deferrals	11
Conclusions	11
APPENDICES	
Appendix I: Flowsheet for Determining Whether a Metabolite Should be Included In the Tolerance Expression	13
Appendix II: Reference Sources	14

DIETARY EXPOSURE BRANCH

STANDARD EVALUATION PROCEDURE

Metabolism in Food Animals: Qualitative Nature of the Residue

INTRODUCTION

If a pesticide use could result in residues in edible tissues of livestock (including poultry), in milk, or in eggs, petitioners for tolerances are required by 40 CFR 158.240 to submit animal metabolism studies. The purpose of these studies is to define qualitatively these residues when livestock have been exposed to pesticides either by ingestion of treated feeds, direct exposure, or premise use. These data are used to determine the total toxic residue (the residue considered to be in need of regulation) in tissues, milk, and eggs. Animal metabolism studies are discussed in Subdivision O of the Pesticide Assessment Guidelines: Residue Chemistry, and further guidance for petitioners is in the residue chemistry Data Reporting Guidelines.

This Standard Evaluation Procedure (SEP) is designed to aid Dietary Exposure Branch scientists in their evaluation of animal metabolism studies. A separate SEP deals with plant metabolism studies, and SEPs are also available for other residue chemistry requirements.

DATA EVALUATION

When Are Metabolism Studies Needed for Food Animals?

Normally, metabolism studies are always required for uses that involve direct treatment of livestock or premise uses. If the use under consideration involves application of a pesticide to a crop, metabolism studies are needed if the raw agricultural commodity (RAC) or a processed byproduct could be fed to animals.

RACs and their processed byproducts are listed in Table II of the Residue Chemistry Guidelines, cited above. This table also specifies which of these might be used as feed items, the animals that could ingest them, and the percentage of the total diet that a feed item could comprise. If a reviewer is uncertain whether a certain RAC or processed commodity can be used as a feed item, (s)he should consult the references on animal feeds listed in Appendix II to this SEP or experts in animal feeds, either from

In certain instances the petitioner may wish to avoid the question of secondary residues by imposing label feeding restrictions. These restrictions are appropriate only if they are practical: the feed item must be under the control of the grower (grower control is also indicated in Table II of the Residue Chemistry Guidelines), and it must not be of such economic importance, for example corn fodder, that the grower would be tempted to feed it even if a label feeding restriction were in place. Questions about the practicality of feeding restrictions should be resolved by contacting experts at the USDA or state extension personnel.

Metabolism studies should represent the type of animal that might be exposed. If an important feed item is involved, this will usually require two studies, one for ruminants and one for poultry. Goats are frequently used for the ruminant studies, and chickens are often used for the poultry studies, though other species may be acceptable. If there are significant differences between the poultry or ruminant metabolism and the rat metabolism study required under the toxicology guidelines, a non-ruminant metabolism study (usually swine) is also required. A "significant difference" means that metabolites uncovered in the ruminant or poultry were not found in the rat, or perhaps the same metabolites were uncovered, but their relative levels were quite different.

Since the route of exposure may affect metabolism, separate studies are usually required for dermal applications and ingestion of treated feed.

Data Requirements

Below is a list of data requirements that should be submitted with animal metabolism studies. Each is accompanied by a discussion of considerations for the reviewer.

1. Information concerning the test material

Metabolism studies are carried out with radiolabeled pesticide. The position of the label, the specific activity, and the radiochemical purity should be provided. There should be sound rationale for the radioactive atom used and its placement in the molecule. For complex molecules that are expected to result in large fragments, more than one label may be needed. Placement of the radiolabeled atom in a portion of the molecule that is expected to be labile, or use of a radioactive atom that is subject to exchange reactions (e.g., tritium), should be questioned.

Generally, parent compound is fed in metabolism studies. Animals should not be fed a mixture of parent and metabolites corresponding to those uncovered in plant metabolism studies. If this

were done, it would be impossible to determine whether a metabolite arose from metabolism of the parent compound or because the animal was fed that metabolite.

Concern over a metabolite found in plants may lead to the requirement of a separate animal metabolism study for that metabolite, but if a metabolite is uncovered in both plant and animal metabolism studies in which the test compound was the parent, a separate animal metabolism study for that metabolite is not needed.

2. Information concerning animals

A brief history of the animals, including their age, weight, health status, and breed, should be submitted. Normally only one animal is required, but for poultry it is often desirable to dose several birds in order to obtain larger samples for easier characterization of residues. Any significant health problems would raise questions about the validity of the study. The petitioner should explain any unusual treatment of the animals.

Occasionally metabolism studies and feeding studies are combined; in these instances the reviewer should make sure that the requirements of feeding studies (number of animals, duration of dosing, etc.; see feeding study SEP) are also met.

3. Dosing

The metabolism study should closely approximate the exposure of pesticide, both in the level and the route. Separate studies are required for dermal use and uses that result in residues in feed items to determine whether different types of exposure result in different metabolic profiles. One purpose of these studies is to identify metabolites, and exaggerated doses may be needed to ensure sufficient radioactivity for characterization.

For oral dosing, the study should be at least 3 days long and involve no preconditioning (feeding unlabeled chemical prior to the dosing period). The type of dosing (for example: capsule; with feed; bolus) and how it was prepared should be described and the dosing should be well documented. The dose should be stated in terms of mg/animal, and the animals' weight and feed consumption should also be given so that the dose can be calculated in terms of mg/kg body weight and ppm in the feed. The raw data and calculations should be included.

Dermal application of a radiolabeled pesticide should reflect the proposed formulation so that absorption will reflect that expected from the proposed use. Animals that have been dermally treated with radiolabeled pesticide should be maintained so that

oral exposure due to grooming is minimized; this is usually done through the use of metabolism chambers. Otherwise, the study may turn out to be an oral metabolism study.

4. Sample collection

Milk and eggs should be collected during the dosing period in a manner that resembles normal practice, generally twice daily. Animals should be sacrificed within 24 hours of the last dose, and samples of muscle, liver, kidney (except poultry), and fat should be stored frozen until analysis. For dermal metabolism studies, skin samples should also be taken. The petitioner should describe the collection and storage procedures and length of storage to establish sample integrity.

Collection of excreta is especially helpful in determining the fate of pesticides that do not appear in tissues. Determining residues in excreta will also establish whether the complete dose has been administered. For some pesticides, activity may be lost as gases, and the use of a closed system such as a metabolism chamber and traps to catch volatiles is useful; use of a closed system such as this would be required only if it is suspected that volatilization prevents a characterizable amount of activity from entering tissues. Failure to collect excreta or volatiles is not necessarily a deficiency, depending on whether the activity in tissues has otherwise been sufficiently characterized. Occasionally, analysis of excreta will provide useful information that is not readily apparent on tissue analysis.

The treatment of the samples (sampling method, storage conditions, was the sample macerated before storage?, etc.) should be described so that the reviewer can determine the condition of the samples during the experiment at any time from sampling to the final characterization of activity. Generally, storage at freezer temperatures for a month or less is acceptable, but some pesticides or pesticide metabolites will break down quickly in tissues, even at freezer temperatures. If short, the half-life of the radioactive atom should be considered. The reviewer should be convinced that storage conditions have not invalidated the petitioner's results; otherwise, additional data or an additional study may be needed.

5. Quantitation of activity

The total radioactivity should be counted prior to any manipulations that may result in loss of activity. Normally the sample is combusted and the resulting $^{14}CO_2$ counted. The sensitivity of the experiment should be stated along with supporting calcula- tions and raw data. Activity should be reported in terms of radioactive counts and ppm (normally in equivalents of parent compound). Radioactive counting data, other raw data (sample sizes, etc.), and calculations should be

Often much of the activity (>95%) will be recovered in excreta. This does not necessarily mean that residues will not accumulate in tissues; thus, the requirements for characterization of tissue activity are the same as if only a small portion of the activity were recovered in the excreta.

6. Extraction of activity

The petitioner should describe the extraction of activity in detail, including the apparatus, solvents, and conditions of extraction. As extractions proceed, the schemes may become complicated by the number of fractions required. The use of a flowsheet, either provided by the petitioner or made up by the reviewer, often describes the procedure more clearly. Any residue not released by extraction should be subjected to acid, base, or enzymatic hydrolysis in an attempt to solubilize it. Water-soluble activity should be hydrolyzed to release conjugates.

The amount of activity in each fraction (e.g., water-soluble, organosoluble, released by hydrolysis, etc.) should be quantified and expressed in terms of radioactive counts, ppm, and percent of total activity in that sample.

It is inevitable that activity will be lost during the extraction process and during the purification of metabolites. These losses are acceptable provided the reviewer is convinced that they do not represent a relatively large portion of the residue that could contain a single metabolite. For example, the reviewer should express concern over a large amount of activity that the petitioner may have discarded from a single fraction of a TLC plate, but if some activity were lost through several manipulations (TLC, extractions, etc.) there usually is not cause for concern.

Based on the extraction data, the reviewer should be able to tentatively classify the radioactive residues. Residues in animals (and in plants) are commonly classified as either free, conjugated, bound, or as natural constituents. These terms are discussed thoroughly in an article by H. W. Dorough (see reference 3, Appendix II), which serves as a basis for the following definitions:

> Free metabolites are extractable by organic solvents. These metabolites are normally organosoluble and have not further reacted with natural constituents of the animal.
>
> Conjugate metabolites are those that have reacted with chemicals in the animal to form water-soluble compounds. The conjugate is made up of two parts: that

derived from the pesticide, called the exocon, and that from the animal, called the endocon. The endocon is often a sugar, but there are other possibilities (e.g., sulfates, amino acids, glutathione). Identification of the exocon is not normally possible without cleavage of the conjugate bond. This is normally done by acid, base, or enzymatic hydrolysis. After hydrolysis the pesticide or pesticide metabolite, free of the conjugating moiety, is usually organosoluble.

Bound metabolites result from pesticide or pesticide metabolites bonding with cellular components to yield products that can not be removed from the matrix by exhaustive extraction with polar and apolar solvents. If these residues are removed chemically, e.g., by acid, base, or enzymatic hydrolysis, a subclass of bound residues must be established. However, the use of severe conditions to release bound residues may significantly alter the pesticide moiety.

Natural constituents is the term applied to a pesticide or pesticide metabolite that is incorporated into normal cell constituents. If soluble, natural constituents may be difficult to distinguish from conjugates and may be misclassified as conjugates. If the natural constituents are unextractable, they are difficult to distinguish from bound metabolites. This may lead to the misclassification of these residues as bound pesticide metabolites, when they are actually natural constituents containing incorporated radioactivity.

It may be desirable to establish that radioactive residues are natural constituents, particularly if these are thought to comprise a large portion of the activity. This is often difficult to do, and should only be required if a significant portion of the residue cannot be released by extraction or hydrolysis. TOX should also be consulted as to the need for these studies. If needed, there are literature references that will serve as guidelines (see references 8 and 9, Appendix II).

7. Characterization and identification of activity

Because approaches to characterization and identification of metabolites may differ, and because special problems will require special solutions, it is not practical to provide specific criteria that must be met. The methods chosen to characterize the activity are the petitioner's; it is the responsibility of the reviewer to ensure that the experiments are scientifically sound and complete.

In general, any single component that comprises more than 10 percent of the terminal residue should be identified; if identification is not possible after a reasonable effort has been made or is not feasible because the absolute amount of activity is low, then the reviewer may defer to TOX as to their concern over the metabolite(s). The deferral should include as much as is known about the material and the maximum level expected as a result of the proposed use (see Deferrals, below).

Metabolites that comprise a small portion (<10%) of the terminal residue should be identified if possible, but if such a metabolite would not be expected to comprise a quantifiable component of the terminal residue, then identification is not necessary. Also, if it can be shown that the metabolite is actually a mixture of metabolites, none of which comprise a significant portion of the activity, then identification is not necessary. Chromatographic evidence that activity is a complex mixture should be carefully examined. The reviewer should be certain that activity spread over a TLC plate does so because it is a mixture and not because co-extractives or poor technique has caused a single component to smear.

Occasionally several minor metabolites are found to contain a common moiety that may be of toxicological significance. If this is a possibility, it is important that the petitioner attempt to convert minor metabolites to a single compound by hydrolysis or other chemical means.

The activity should be identified to the degree possible. Mass spectral data will generally stand by itself in identification of a metabolite. Other definitive methods (e.g., NMR) often require too much material to be useful in metabolism studies.

The limitations of chromatographic techniques as tools of identification should be borne in mind as metabolism studies are reviewed. Identification by thin layer chromatography (TLC) should be confirmed by a second method or a second TLC system. If TLC data are not compelling, the identity of the metabolite should be confirmed by mass spectroscopy. Any reasonable doubt about the identity of a metabolite constitutes a data gap, and the petitioner should be required to confirm their findings.

The activity in each tissue and in milk and eggs should be accounted for as either:

a. Identified; its name, structure, and level (in ppm as well as a percentage of the total activity in that tissue) should be given.

b. Characterized short of identification; the chemical characteristics (solubility, behavior on treatment with acid, base or enzyme, etc.) and its level in

ppm and as a percentage of the total activity in that tissue should be given. Any residue that is not lost is at least partially characterized by virtue of knowing its solubility (or lack of solubility).

c. Lost; losses should be explained as fully as possible, and the amount of activity lost should be given as a percentage of the total activity and in ppm (parent equivalents).

8. Raw data

Raw data for counting radioactivity should be submitted, along with sample calculations of residue levels. Reproductions of TLCs, GLCs, HPLCs, mass spectra, etc. should be submitted. It is not usually necessary to submit all raw data, but there should be enough to support any identification of metabolites. Detailed accounts of operating conditions for analytical instruments may be desirable, but these do not normally bridge the gap between marginal and certain identification, and their omission does not necessarily constitute a data gap.

9. Expression of a tolerance

The parent compound and significant metabolites are normally included in the tolerance expression. "Significance" depends on 1) whether TOX expresses concern over the metabolite, 2) what percentage of the terminal residue the metabolite comprises, and 3) the absolute level of the metabolite. If TOX expresses no concern because the metabolite is considered innocuous, then it need not be included in the tolerance expression unless it comprises a significant portion of the residue.

If a metabolite is related to the parent and of no special toxicological concern, and its regulation would affect (raise) the tolerance level, then it should be included in the tolerance expression.

Whether or not a metabolite should be included in the tolerance expression also depends on the availability of enforcement methodology; if no enforcement method can be developed for a particular metabolite, then its inclusion in the tolerance may not be necessary. However, if the metabolite is of toxicological significance (as determined by TOX) and, in addition, constitutes a large portion of the residue, then it requires regulation and appropriate methodology.

The possibility of using an indicator compound for the tolerance expression should be considered if one compound (parent or metabolite), or a common moiety to which two or more metabolites can be converted, represents a large and relatively consistent portion of the terminal residue. This cannot be determined until

feeding studies in which all the residue of concern is determined are completed and evaluated. Under the indicator compound concept, only one compound is regulated under the tolerance expression, and the remaining residue of concern is estimated based on feeding studies.

In Appendix I there is a flowsheet designed to aid in the determination of whether or not a metabolite needs to be regulated. It does not cover every possible situation, and several questions in the flowsheet can be answered only with the help of TOX, but its use should help ensure consistency in the way metabolites are regulated.

WRITING A REVIEW

The purpose of these studies is to determine the terminal residue in animals exposed to pesticides. There is more than one acceptable approach to solving this problem. Two questions should be brought up constantly as these studies are being evaluated: 1) Are the experiments performed by the petitioner scientifically sound? and 2) Would additional experimentation add significantly to the results? If the reviewer cannot confidently make a conclusion as to the nature of the terminal residue, then additional studies or data are needed.

An initial perusal of the study provides an opportunity to list deficiencies and make comments. This will also provide an outline to serve as a basis for the complete review. The reviewer should constantly compare the raw data with the petitioner's conclusions; any wrong or questionable interpretation of the data constitutes a data gap, and supplementary data or perhaps additional experimentation should be required. Comparison of the study with previously reviewed studies on the same or similar chemical(s) may be useful in interpreting the data.

The written report of the reviewer should include all the information that is the basis for conclusions or deficiencies. For clarity, extensive numerical data should be listed in tabular form. If a particular experiment or part of an experiment is found to be deficient, the reviewer should spell out in detail what is required of the petitioner. If the petitioner has failed to submit any of the data requirements, it should be listed as a deficiency. If the data submitted are deficient or unclear to the extent that the reviewer does not feel comfortable in making conclusions about the metabolism, then clarification or further work should be required.

A list of references that may be useful as an aid in the review of metabolism studies is given in Appendix II. In addition, the reviewer should make use of USDA and extension personnel, particularly to answer questions about agricultural practices.

Flexibility on the part of the reviewer may be appropriate for certain uses. For example, uses that result in low residues in a minor feed item (and in no other feed items) may not require as definitive a metabolism study as would be required for a use that resulted in relatively high residues in an important feed item. However, the metabolism of any pesticide whose potential exposure to food animals is significant, either because of being present in an important feed item or in several less important feed items, must be adequately understood.

Deferrals

The decision as to whether metabolites should be regulated often requires help from the Toxicology Branches. Deferrals should be as specific as possible. If a metabolite is identified, its relationship to the parent compound should be made clear. If unidentified, as much information as is known should be included in the deferral.

Deferrals to TOX are appropriate in the following situations:

1. After repeated efforts, a significant portion of the sidue remains unidentified. TOX should be given as much information as is available about this material and asked whether their concern warrants identification or whether the partial characterization will suffi

2. An identified metabolite constitutes greater than 10% of the total residue. TOX should be asked whether this material is required to be included in the tolerance expression. The 10% should be considered a guideline; the absolute level expected should also be provided to help TOX make a decision. TOX may be concerned about a metabolite when its expected level rises to a certain point; thus, it may be necessary to defer to TOX several times concerning the same metabolite as the uses of a pesticide increase.

Deferrals can be time-consuming and should be avoided if possible. It may be feasible to resolve questions by consulting other DEB reviewers or by making an oral deferral to TOX, though matters of importance require a written deferral.

Conclusions

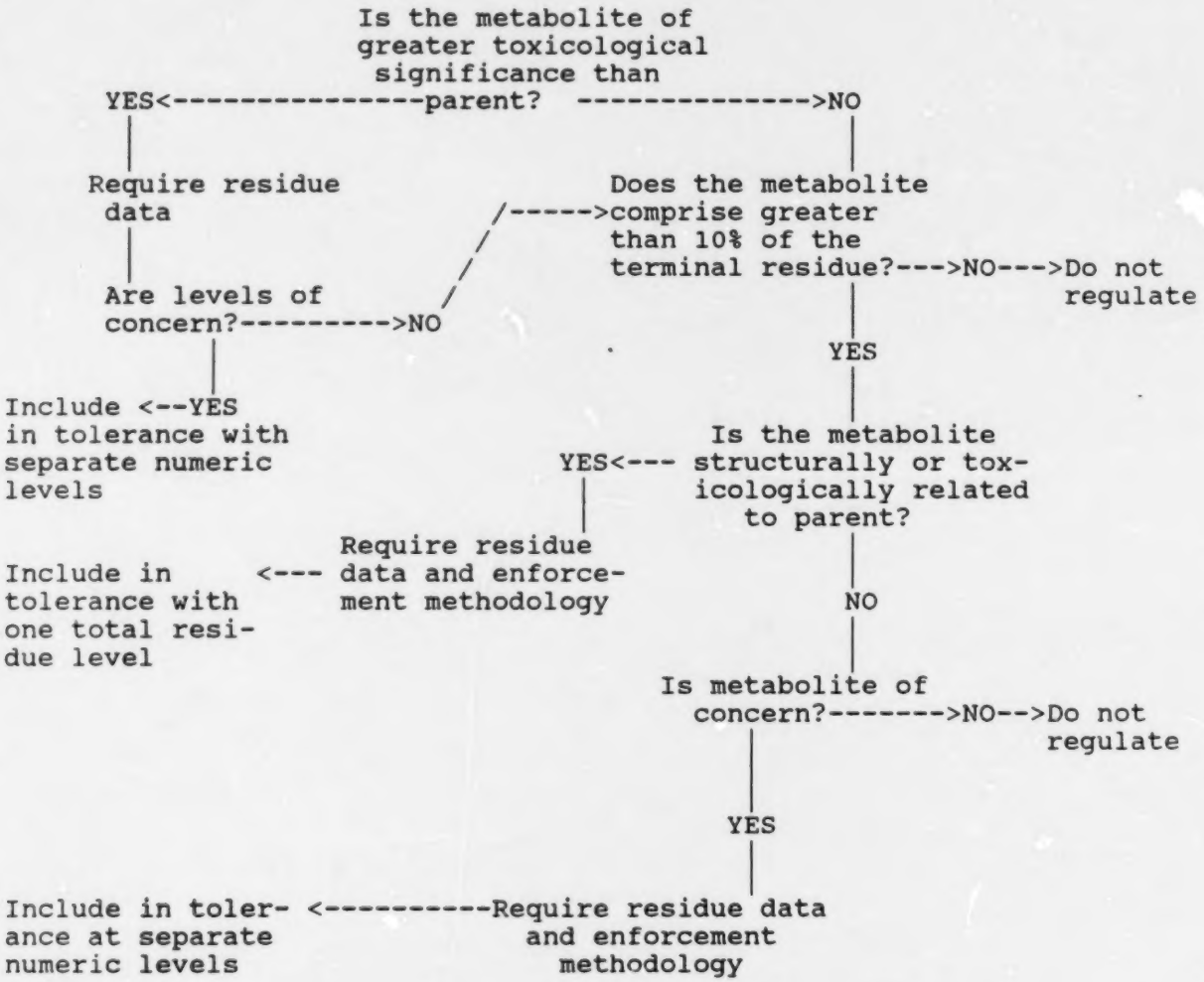
After reviewing an animal metabolism study, the reviewer should state what is understood about metabolism in the subject animal, what, if any, additional animal metabolism data or clarification of existing data is needed to support the proposed use, and, if the metabolism is adequately understood, a conclusion as to what

components of the residue are in need of regulation. If this latter conclusion cannot be made without the assistance of TOX, a deferral should be made.

If future uses may or will require additional metabolism studies, this should be made clear in the conclusions.

APPENDIX I

Flowsheet for Determining Whether a Metabolite Should be Included in the Tolerance Expression


Is the metabolite of greater toxicological significance than parent?
YES
NO
Require residue data
Are levels of concern?
NO
Include in tolerance with separate numeric levels
Does the metabolite comprise greater than 10% of the terminal residue?
NO
Do not regulate
YES
Is the metabolite structurally or toxicologically related to parent?
YES
Require residue data and enforcement methodology
Include in tolerance with one total residue level
NO
Is metabolite of concern?
NO
Do not regulate
YES
Require residue data and enforcement methodology
Include in tolerance at separate numeric levels

APPENDIX II

Reference Sources

1. Pesticide Assessment Guidelines: Subdivision O: Residue Chemistry, prepared by OPTS/EPA, 1982.

2. Menzie, C.M., Metabolism of Pesticides (including three updates).

3. Dorough, H.W., "Classification of Radioactive Pesticide Residues in Food Producing Animals," J. Environ. Path. and Tox., 3, 11-19, (1980).

4. Directory of Professional Workers in State Agricultural Experiment Stations and Other Cooperating State Institutions, USDA Agricultural Handbook No. 305, 1984.

5. "Guidelines for Data Acquisition and Data Quality Evaluation in Environmental Chemistry," Analytical Chemistry, 52, 2242 (1980).

6. Food and Feed Crops of the United States, J. R. Magness, G. M. Markle, and C. C. Compton, Rutgers University, N.J., 1971.

7. Feeds and Feeding, Abridged: The Essentials of the Feeding, Care, and Management of Farm Animals, Including Poultry, F. B. Morrison, 9th Edition, The Morrison Publishing Company, Ithaca, N.Y., 1958.

8. Hutson, D.H., and Hoadley, E.C.: The comparative metabolism of ^{14}C-vinyl-dichlorovos in animals and man, Arch. Toxikol., 30, 9-18, 1972.

9. Dorough, D.H., and Ivie, G.W.: Carbon-14 milk constituents resulting from feeding carbonyl-^{14}C carbamate to cows, Science, 159, 723-733, 1968.